

The composition of trigeminal nerve branches in normal adult chickens and after debeaking at different ages

J. L. DUBBELDAM, M. A. G. DE BAKKER AND R. G. BOUT

Neurobehavioral Morphology, Institute of Evolutionary and Ecological Sciences, Leiden University, The Netherlands

(Accepted 3 January 1995)

ABSTRACT

The long term effects of amputation of the tip of the beak were studied in adult hens that were debeaked on the day of hatching, at the age of 8 d and at 6 wk, by EM analysis of fibre spectra of the medial branch of the ophthalmic nerve and of the intramandibular nerve. Three categories of fibre were distinguished for further analysis, i.e. unmyelinated axons, small myelinated fibres and large myelinated fibres. In normal birds the ophthalmic nerve contains relatively more large fibres than the intramandibular nerve. Amputation consistently results in a reduction of the number of large fibres and a substantial increase in the number of small myelinated fibres. The proportion of unmyelinated axons is rather variable, but is not affected by beak trimming. Age at debeaking has no effect. The observations are inconclusive concerning the possibility of heightened nociception.

Key words: *Gallus domesticus*; peripheral nerve; axotomy.

INTRODUCTION

Debeaking or beak trimming, i.e. removal of the tips of the beaks, is a widespread practice in large-scale housing of laying hens. The main reason for this practice is the prevention of cannibalistic pecking. From studies in other birds it is known that the tips of beaks contain considerable numbers of mechanoreceptors and free nerve endings and are well innervated (Berkhoudt, 1980; Gottschaldt, 1985). More recent observations make it clear that this is also true for the chicken (Desserich et al. 1983; Gentle & Breward, 1986; Gentle, 1989). Debeaking not only removes these nerves and sense organs, but may also give rise to neuromas (Desserich et al. 1984, Breward & Gentle, 1985). Debeaking may thus be less harmless than is sometimes assumed. This tentative conclusion is supported by Breward & Gentle (1985), who reported an increase in spontaneous activity in trigeminal branches after debeaking. With the exception of these observations, relatively little is known about the effect of amputations on the development of the trigeminal system in birds. For this reason we

initiated a series of observations on this system in normal chickens and following early debeaking. The birds used in this study are a small sample of a large number of animals that had previously been used to estimate the effects of beak amputation under different conditions and at different ages on the appearance, growth and well-being of the animals (Research Project of Centre of Poultry Research 'Het Spelderholt'; see Van Rooyen, 1990; Van Rooyen & Blokhuis, 1990). In this communication the results of an electron microscope study on the fibre spectra of trigeminal branches are reported with emphasis on the long term effects of amputation. The effects within the central nervous system were the subject of a complementary study (Dubbeldam & Den Boer-Visser, 1993).

MATERIAL AND METHODS

All adult hens (aged 20–25 wk) used for the EM analysis were obtained from the Centre for Poultry Research and Information Services 'Het Spelderholt'. Four categories of animals were used: birds with

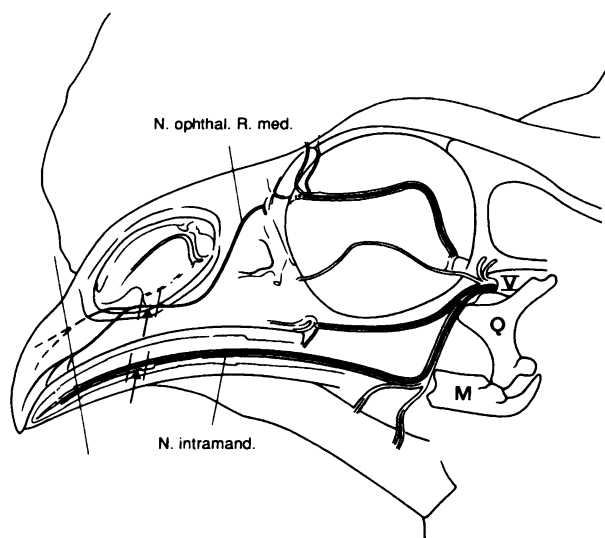


Fig. 1. Outline of chicken head to indicate where samples from the medial ophthalmic and intramandibular nerves were taken (arrows). The site of beak amputation is indicated by the vertical line. M, mandible; N. intramand., intramandibular nerve; N. ophthal. R. med., medial branch of the ophthalmic nerve; Q, quadratum; V, (bony capsule of) trigeminal ganglion.

normal beaks and birds debeaked on day (d) 1 after hatching, on d 8 or after 6 wk. Beak trimming was performed with a debeaking apparatus using a heated knife (650–800 °C). Untreated animals of the same strain were used for comparison. All amputations were performed in 'Het Spelderholt' by a professional 'debeaker'. In all animals rather large portions of the upper and lower beak were removed (Fig. 1). They survived at least until the laying had started.

The animals were deeply anaesthetised using pentobarbitone sodium (Nembutal) and were perfused through the heart with about 1 l saline (0.75% NaCl in distilled water) + 2 ml heparin added per l solution; this was followed by perfusion through the carotid arteries with about 200 ml of 1.5% glutaraldehyde + 1% paraformaldehyde in 0.075 M cacodylate buffer, pH 7.2.

Small portions from the medialis branch of the ophthalmic nerve in the upper beak (just in front of the nares) and from the intramandibular branch of the mandibular nerve in the lower beak (just after its ventral curvature; Fig. 1) were dissected out, cut into pieces about 2 mm in length and postfixed in 1% OsO_4 in cacodylate buffer for 4 h. Potassium ferrocyanide was added to the fixation fluid (at a concentration of 4 mM) to enhance membrane staining. The tissue was dehydrated and embedded in Epon. Sections (80 nm) were cut on a Reichert–Jung Ultracut E ultratome, collected on 200 mesh grids and contrasted with uranyl citrate and lead nitrate. The

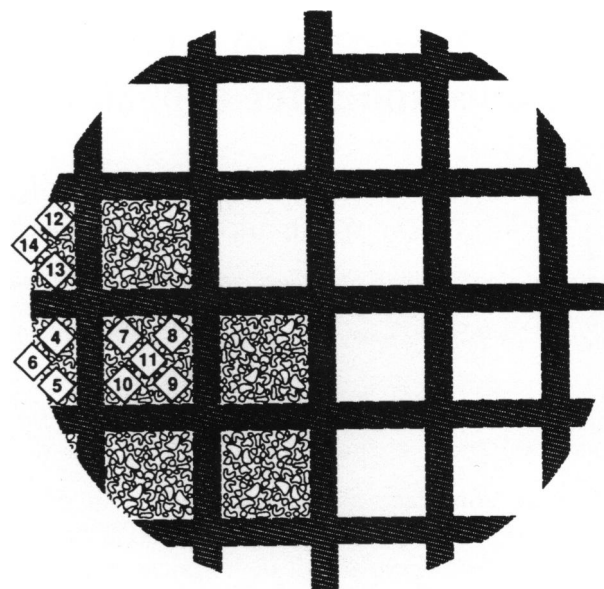


Fig. 2. Drawing showing the distribution of the photographs that were used for measurement.

sections were inspected and photographed using a JEOL JEM-100CXII transmission electron microscope. The grid bars were used to select the areas for photography. In each grid square 5 pictures were taken, 1 in each corner and a 5th one in the centre (Fig. 2). For the medial branch of the ophthalmic nerve, 3 films of 38 exposures each were used; for the intramandibular nerve a single film was sufficient. The first and last exposure of each film was a picture of a standard grid with 1200 lines per mm. These were used to calculate the final magnification of the photographs.

The surface area of the myelinated fibres (axon + myelin sheath) was used as a measure of their size. The cross-sections of all complete fibres on a photograph were digitised using an x–y tablet (Calcomp 2500) connected to a Commodore PC20 and their surface areas calculated. All unmyelinated axons were counted in each photograph, but not further subdivided into size classes. Light microscopical photographs were used to estimate the total area of the cross section of each nerve. The total numbers of myelinated and unmyelinated fibres in each nerve were calculated from the ratio of sampled and total nerve area.

The sampling technique used in this study may result in an underestimation of the number of large fibres, as the chance of falling only partly within the frame of the photograph is greater for large than for small fibres. We estimated the surface area of the photographs which was effectively sampled by subtracting the average diameter of axon + myelin sheath from the dimensions of the photographs for 9 size

Table 1. Numbers of fibres counted in microphotographs of ophthalmic and intramandibular nerves in normal and debeaked animals

		Myelinated			Area measured (%)	Total surface area (μm²)
Code	Unmyelinated	< 26 μm²	> 26 μm²			
Normal						
L opht	Za	212	263	219	21.5	310782
L opht	Z9	426	297	234	21.8	280933
L opht	Z10	268	220	229	22.8	268934
L mand	Za	123	137	81	23.7	97610
L mand	Z9	382	289	139	29.2	119241
L mand	Z10	437	269	161	31.8	123711
R mand	Za	135	237	134	28.1	95267
R mand	Z11	201	335	135	32.7	91642
Day 1						
L opht	Z6	419	343	228	24.0	278124
L opht	Z7	620	671	163	23.4	181381
L mand	Z2	294	343	90	34.4	86275
L mand	Z6	207	247	86	25.0	143464
L mand	Z7	647	543	65	30.1	109425
R mand	Z6	386	370	131	36.9	85959
R mand	Z7	505	474	97	31.4	104163
Day 8						
L opht	Z3	171	367	133	26.4	132667
L mand	Z3	232	355	63	24.9	81697
R mand	Z3	261	335	46	22.6	85915
R mand	Z8	318	395	65	22.6	106561
Week 6						
L opht	Z0	409	724	111	27.4	262183
L opht	Z12	352	644	208	23.5	256697
L mand	Z0	192	399	91	24.8	110774
L mand	Z4	183	286	42	24.0	102565
L mand	Z12	595	583	88	31.2	112317
R mand	Z4	185	253	75	28.7	61095

L, left; R, right; opht, ophthalmic nerve; mand, intramandibular nerve.

classes (interval 13 μm^2). From these corrections on the sample area we calculated that the number for the class of myelinated fibres $\leq 26 \mu\text{m}^2$ should be corrected by $\times 1.25$ and that for myelinated fibres $\geq 26 \mu\text{m}^2$ by $\times 1.75$. These corrections were carried through in the calculations of the 'absolute numbers' of fibres in the respective size classes (Table 2).

A total of 17 intramandibular nerves and 8 ophthalmic branches collected from 13 animals were used in the comparisons. For the distribution over the categories, see Table 1.

RESULTS

The medial branch of the ophthalmic nerve innervates the mechanoreceptors in the upper beak and the intramandibular branch those of the lower beak. In all sections, large and small myelinated fibres and clusters of unmyelinated axons were found throughout the section (Fig. 3).

In a first approach the cross sectional areas of the fibres were measured both without and with myelin sheaths. For 480 fibres of the ophthalmic nerve and 218 fibres of the intramandibular nerve, all from 1 intact animal, the cross-sectional areas of axons and of myelin sheaths appear to be highly correlated ($r = 0.865$). Over part of the range the data may be described by a linear relationship. For small fibres ($\leq 26 \mu\text{m}^2$, the largest proportion of fibres) the myelin area is roughly 1.8 times the axon area (straight line in Fig. 4). However, this relationship systematically overestimates the myelin area of the largest axons. Even though the large axons are relatively scarce, the data may be better described by the relationship: myelinated area = $-12.56 + 10.84 \text{ axon area}^{0.49}$ (curved line in Fig. 4). This function suggests a negative allometric relationship; myelin area approaches zero at an axonal area of 1.36 μm^2 . The largest unmyelinated fibres in our sample had an area of 2.05 μm^2 and the smallest myelinated fibres an area of 0.35 μm^2 .

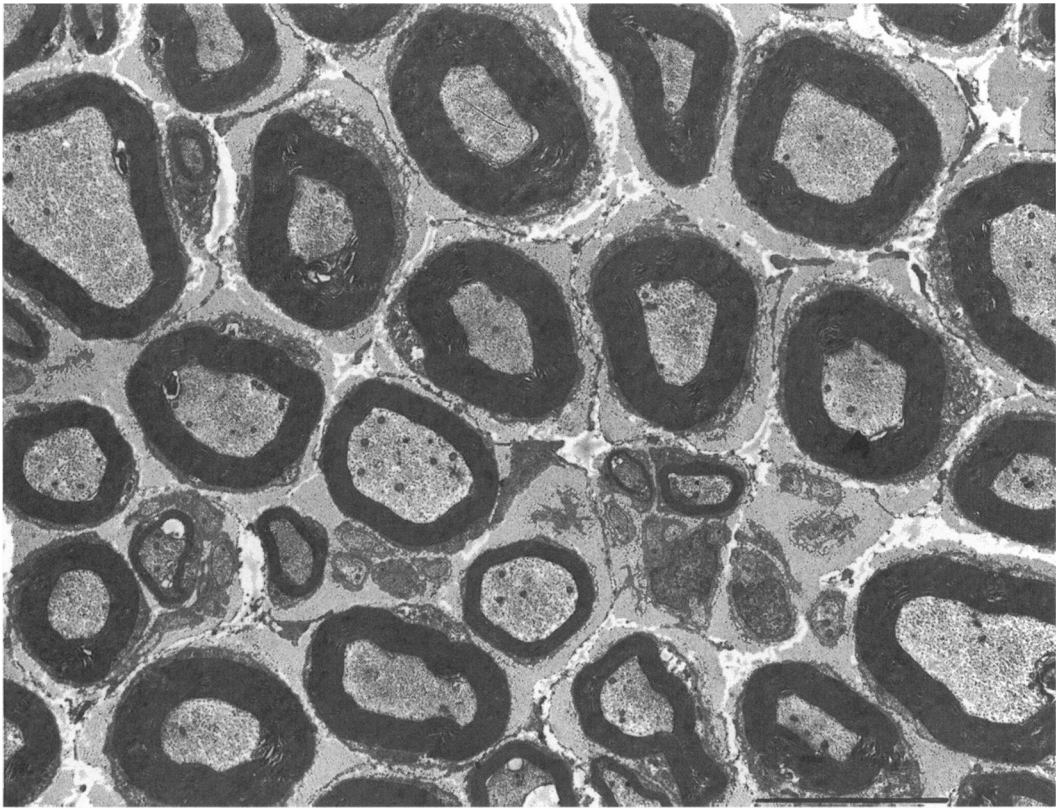


Fig. 3. Electron micrograph of part of a cross section of the intramandibular nerve of a bird debeaked on d 8. Bar, 5 μm .

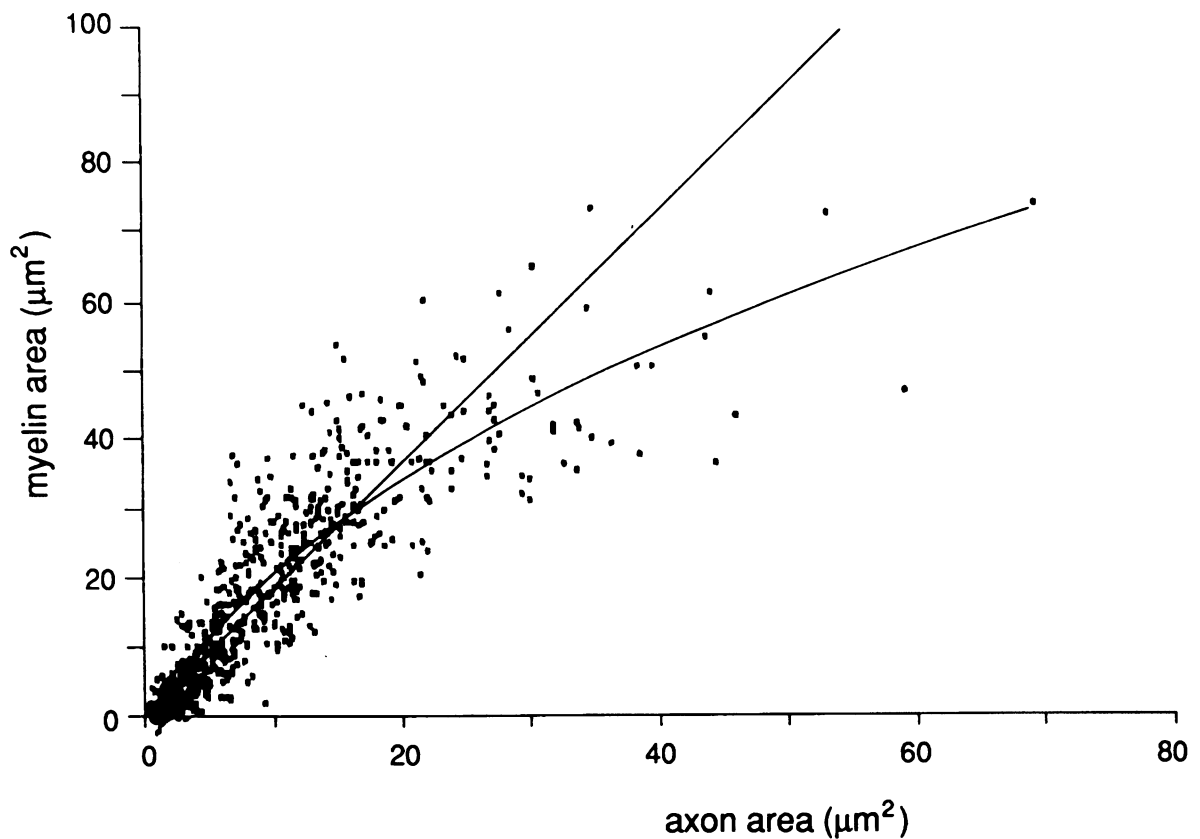


Fig. 4. Relationship between axon area and myelin area for a sample of ophthalmic and intramandibular fibres from a normal chicken. For further explanation, see text.

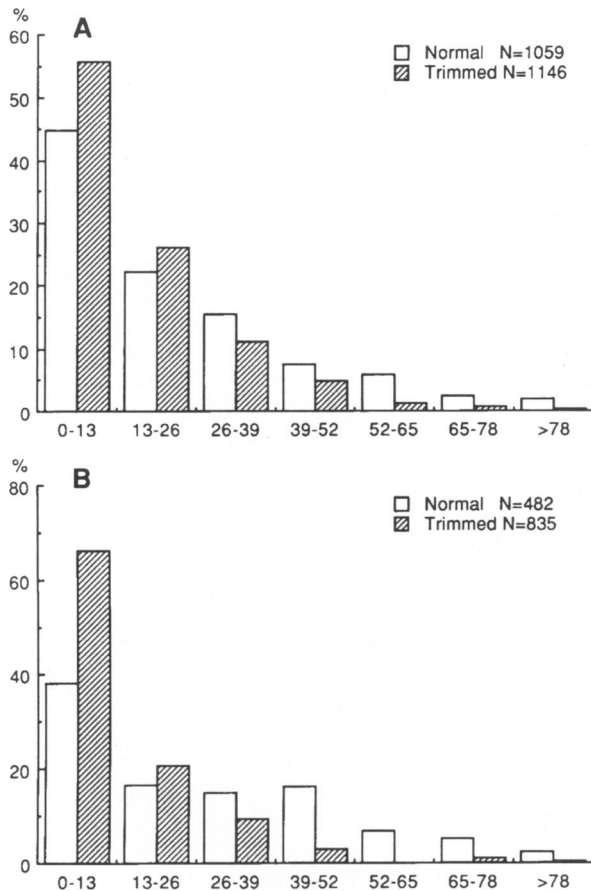


Fig. 5. Histograms of the classes of myelinated fibres in the intramandibular nerve (A) and ophthalmic nerve (B) in a normal bird and after debeaking at the age of 6 wk.

Only the measurements of the cross-sectional areas of the axons together with their sheaths were used for further analysis. Figure 5 shows a typical example of the distribution of myelinated fibres over the different size classes for both the ophthalmic and intramandibular nerves in a normal bird and in a bird debeaked at the age of 6 wk. The distributions are highly skewed towards the small calibre fibres. In all 3 birds the distribution of fibres in the left intramandibular nerve was significantly different from the distribution in the left ophthalmic nerve (χ^2 test; $P < 0.01$). The intramandibular nerve contained relatively more small fibres. There was also a distinct difference between intact and debeaked animals (both beaks $P < 0.001$): there was a relative increase in the number of small fibres (i.e. $\leq 26 \mu\text{m}^2$) in both nerves (Table 2). A similar effect was seen after debeaking of younger animals. Therefore, for further analysis, fibres were subdivided into 3 classes, i.e. unmyelinated axons, and small ($\leq 26 \mu\text{m}^2$) and large ($\geq 26 \mu\text{m}^2$) myelinated fibres. The results from all measurements are compiled in Table 1. For all nerves the total

number of fibres and the percentages of each size class were calculated (Table 2). The mean numbers of fibres were calculated for each category of bird; in these calculations the d 1 and d 8 birds were combined. Thus 3 categories of birds were compared, i.e. with normal beaks, after early debeaking and after late debeaking.

Notwithstanding a clear interindividual variation, some general trends could be recognised. There was a significant decrease of the number of large fibres in the intramandibular nerve (ANOVA; $F_{[2,14]} = 11.67$, $P < 0.01$). A comparable (although nonsignificant) trend was seen in the ophthalmic nerve. In both nerves, the reduction in the number of large fibres was accompanied by a distinct increase in the number of small myelinated fibres (ophthalmic: $F_{[2,5]} = 5.70$, $P < 0.05$; mandibular: $F_{[2,14]} = 4.57$, $P < 0.05$). The numbers of unmyelinated axons were highly variable within each category, but there was no indication of an increase of this class of fibres after beak trimming. The net effect seems to have been an increase in the total numbers of fibres after debeaking: 5304 (d 1–8) and 5975 (wk 6) vs 4000 (normal) for the ophthalmic nerve, 3447 (d 1–8) and 3230 (wk 6) vs 2690 for the intramandibular nerve. The age at debeaking appeared to have no effect on fibre composition.

DISCUSSION

The central question at the start of this study was whether it is possible to use morphological criteria to assess the severity of damage after beak trimming. To answer this question it is necessary to know what morphological changes occur in that portion of the trigeminal system that loses its peripheral innervation area. The main part of the present investigation concerned possible changes in the peripheral nervous system. After considering the problem of morphological changes, we will survey what evidence exists that the normal behaviour of the birds is affected as a result of beak trimming.

The composition of the intramandibular and medial ophthalmic nerves in normal birds and after debeaking

The main body of our data concerns the composition of the 2 trigeminal branches. We assume that the intramandibular nerve contains mainly sensory fibres, as sympathetic fibres innervating salivary glands branch off more caudally with the sublingual branch (cf. Dubbeldam, 1993). The medial ophthalmic nerve

Table 2. Calculated absolute numbers and (percentages) of fibres in three size classes in ophthalmic and intramandibular nerves

		Unmyelinated		Myelinated	
	Code	N	(%)	< 26 μm² (%)	> 26 μm² (%)
Normal					
L opht	Za	986	(22.9)	1530 (35.6)	1783 (41.5)
L opht	Z9	1260	(35.3)	1097 (30.8)	1211 (33.9)
L opht	Z10	1174	(28.4)	1205 (29.3)	1755 (42.5)
	Mean	1140	(28.5)	1277 (31.9)	1583 (39.6)
Day 1–8					
L opht	Z6	1745	(33.6)	1785 (34.4)	1661 (32.0)
L opht	Z7	2650	(35.6)	3585 (48.1)	1220 (16.4)
L opht	Z3	647	(19.8)	1736 (53.2)	880 (27.0)
	Mean	1681	(31.7)	2369 (44.7)	1254 (23.6)
Week 6					
L opht	Z0	1468	(27.1)	3289 (60.0)	705 (12.9)
L opht	Z12	1497	(23.1)	3425 (52.9)	1549 (23.9)
	Mean	1491	(25.0)	3357 (56.2)	1127 (18.8)
Normal					
L mand	Za	519	(28.2)	723 (39.3)	599 (32.5)
R mand	Za	467	(20.3)	1024 (44.5)	810 (35.2)
L mand	Z9	1306	(38.7)	1235 (36.6)	831 (24.7)
L mand	Z10	1376	(41.4)	1059 (31.9)	887 (26.7)
R mand	Z11	614	(23.8)	1280 (48.9)	723 (27.6)
	Mean	856	(31.8)	1064 (39.6)	770 (28.6)
Day 1–8					
L mand	Z2	855	(33.4)	1246 (48.7)	458 (17.9)
L mand	Z6	829	(31.0)	1237 (46.3)	604 (22.6)
R mand	Z6	1045	(35.8)	1251 (42.9)	621 (21.3)
L mand	Z7	2148	(44.9)	2254 (47.1)	378 (7.9)
R mand	Z7	1607	(39.9)	1885 (46.7)	541 (13.4)
L mand	Z3	932	(29.5)	1784 (56.5)	443 (14.0)
R mand	Z3	1154	(34.3)	1851 (55.6)	355 (10.6)
R mand	Z8	1409	(34.4)	2187 (53.3)	504 (12.3)
	Mean	1247	(36.2)	1712 (49.7)	488 (14.1)
Week 6					
L mand	Z0	773	(22.6)	2008 (58.5)	640 (18.7)
L mand	Z4	763	(40.3)	2337 (56.0)	494 (10.4)
R mand	Z4	644	(29.8)	1490 (49.3)	306 (12.0)
L mand	Z12	1909	(29.3)	1100 (50.0)	457 (20.7)
	Mean	1022	(31.6)	1734 (53.7)	474 (14.7)

Abbreviations as in Table 1.

may contain a modest number of sympathetic fibres innervating the most rostral glands in the upper beak. These glands seem not to be affected by the amputation.

Three size classes of fibres were distinguished, unmyelinated axons and small and large myelinated fibres (Table 2). It can be questioned whether the cross-sectional area of axon + myelin sheath is a suitable measure of fibre composition. In a study of the oculomotor nerves in the rat, Fraher (1989) argued that the axon diameter to fibre diameter ratio (g ratio) differs considerably between axons of different size classes. In other studies 2 populations of myelinated fibres have been recognised in sensory as well as in mixed nerves (e.g. Fraher et al. 1990). One

population consists of fibres with small axon diameters and relatively thin myelin sheaths, the g ratio being relatively high. The other population comprises larger axons with thicker myelin sheaths, but these become relatively thinner with increasing axon diameter. In our material also, the g ratio provided a nonlinear measure for the amount of myelination. For the negative allometric relationship fitted to our data the g ratio decreased from 1 (unmyelinated) to about 0.55 ($\sim 5 \mu\text{m}^2$ axon area) and then slowly increased again to 0.7 for the largest fibres in our sample. This relation between g ratio and axon diameter is comparable to that described by Fraher et al. (1990) in the rat. Cross-sectional area as a measure of size deals with the problem of irregular shapes of cross sections

and at the same time provides a more sensitive (i.e. squared) measure than linear dimensions. Our measurements show that for most fibres the relation between the cross-sectional area of axons and the surface area of the myelin sheath is approximately linear. The relative decrease in myelin area of large fibres only affects the 'tails' of the fibre size distribution, making these less elongated. This has little effect on the results of our analysis using the 3 fibre size classes.

Comparing the nerves of the upper and lower beak in normal birds, the ophthalmic nerve appeared to contain a slightly higher proportion of large fibres. This may reflect the presence of a more elaborate bill tip organ in the upper than in the lower beak. The percentage of unmyelinated axons in the 2 nerves (varying between about 23 and 41 % in normal birds) is lower than that found in the frog (65 %) and in various mammals (40–50 %), including man (Young, 1977). In birds as well as in mammals the largest proportion of myelinated fibres are small-size fibres (diameter 2–3 μm , corresponding to our class of fibres smaller than 13 μm^2 ; Fig. 5). A significant loss of large fibres was found in the intramandibular branch; the same trend was seen in the ophthalmic nerve (Table 2). Preliminary observations in the trigeminal ganglia of normal and debeaked chickens suggested a reduction of about 20 % that is mainly due to a smaller number of large cells after debeaking. The proportion of cells larger than 1200 μm^2 was reduced from 20 % to 14 % (Van der Noorda, unpublished observations). This shift seems to reflect the reduction of the number of large fibres.

In a study on degeneration in the trigeminal ganglia of the rat after interruption of the infraorbital nerve, Arvidsson & Aldskogius (1977) and Aldskogius & Arvidsson (1978) reported a loss of about 14 % of the ganglion cells. With about 60 % of the fibres passing through the infraorbital nerves these authors estimated the net loss of damaged cells at 17–29 %. This is of the same order as that found in the chicken. Savy et al. (1981) estimated a cell loss of about 36 % in the ophthalmic-maxillary part of the ganglion in the mouse after destroying the vibrissae follicles at birth. The decrease of the neuronal cell body volume was about 55 %; this suggests a loss of, in particular, large cells. Fried et al. (1991), however, reported a shift in cell characteristics rather than a change of cell numbers. Holland & Robinson (1990) found no or little loss of cells after disruption of the inferior alveolar nerve in the cat. They suggested that changes in the neurons caused by the injury may result in an apparent increase of size and thus mask a loss of cells.

Finally, the cell loss may be more severe when the nerve is damaged in neonatal animals (Himes & Tessler, 1989).

Our observations were based on counts of axons. The calculated 'absolute numbers' showed an increase in all debeaked animals. There was no clear age effect. This increase – for the 2 nerves together from 4000 + 2690 (normal) to 5304 + 3447 (d 1–8) and 5975 + 3230 (wk 6) – seems to contradict the decrease in the number of ganglion cells. A possible explanation of the greater numbers of unmyelinated and small myelinated fibres could be the occurrence of strong collateral branching after debeaking. In mammals regenerated myelinated axons can support more than one process distal to the injury site (Lisney, 1989). Renehan et al. (1989) reported a relative increase in the number of nociceptive afferents after transection of the infraorbital nerve in the adult rat. In our chickens this could be true both for myelinated and unmyelinated fibres; however, our samples were taken proximal to the injury (i.e. the amputation site) and not distal as in most mammalian experiments. A reduction of size of all damaged axons and cells as suggested in mammals (Lisney, 1989) could explain the relative shift in the composition of the nerve, but not the increase in the absolute numbers of fibres. We have seen no indications of the occurrence of recurrent fibres after debeaking but cannot exclude this possibility. In conclusion, our analysis showed a change in composition of the nerve branches caused by a loss of large myelinated fibres and an increase in the number of small myelinated fibres.

Neuroma formation, increased nociceptive sensitivity and loss of the sense of touch

Nerve transection can result in peripheral sprouting (Chiaia et al. 1988) and thus in the development of neuromata (foci of arrested growth; Fried et al. 1991). The development of neuromata has been described in the upper and lower beak after beak trimming by Breward & Gentle (1985). They also reported the occurrence of abnormal patterns of discharge of nociceptive units as well as abnormal spontaneous activity in other units. The latter observations, however, concerned birds that were already adult at the time of debeaking. Histological inspection of some beaktips from our material provided no evidence for the presence of neuromata. Fried & Devor (1988) followed the process of neuroma formation in the adult rat over a longer period and found that in the long term the initial swellings disappear. This may explain the difference between our observations (long-

term effects) and those of Breward & Gentle (survival time from 1 h to 30 d). Fried et al. (1991) reported that tooth extraction in rats or pulpectomies do not regularly result in obvious terminal axonal abnormalities or local neuroma-like formations. Instead the pulp nerve fibres either disappear or possible reinnervate novel targets. In the long term, neuroma formation may thus be less relevant and spontaneous activity may decrease again to a more normal level (Fried & Devor, 1988). In our material all animals survived for at least 26 wk after debeaking.

What remains is a high density of innervation, particularly by small myelinated and unmyelinated fibres, i.e. probably predominantly representing A delta and C fibres. They may therefore include 'nociceptive' fibres and thus a higher susceptibility for pain might be anticipated. Preliminary behavioural observations in our laboratory using acetic acid as a painful stimulus (Dubbeldam et al. 1993) provided at best conflicting results concerning a possibly heightened sensibility: all except 1 normal or debeaked bird refused to accept food soaked in dilute acetic acid at the same concentration, the exception being a debeaked bird. In this instance the painful experience could have been due to damage of the skin of the remaining part of the beak.

Histochemical observations on the distribution of neuropeptides in primary sensory trigeminal nuclei (Dubbeldam & Den Boer-Visser, 1993) did not reveal differences in the intensity of staining in these nuclei. This is a further indication that beaks are not abnormally vulnerable to painful stimuli after debeaking.

There can be little doubt as to the loss of tactile sense ('touch'). Laminated (Herbst) corpuscles – numerous in normal beaks – are completely lacking in the affected region. This loss of end organs may be related to the distinct reduction of large fibres in the ophthalmic and intramandibular branches. Preliminary observations suggested that the reduction of the number of large ganglion cells after debeaking is accompanied by a reduction in volume of the principal sensory trigeminal nucleus (unpublished observations). The central projections and histochemical characterisation of primary nuclei are to be the subject of further study.

ACKNOWLEDGEMENTS

We thank Drs H. J. Blokhuis and J. van Rooyen of 'Het Spelderholt' for stimulating discussions during the course of this project. This study was supported financially by the Commission for Welfare and

Animal Husbandry. Ms Hanna Schut provided essential secretarial support.

REFERENCES

- ALDSKOGIUS H, ARVIDSSON J (1978) Nerve cell degeneration and death in the trigeminal ganglion of the adult rat following peripheral nerve transection. *Journal of Neurocytology* **7**, 229–250.
- ARVIDSSON J, ALDSKOGIUS H (1977) Retrograde neuronal degeneration in the trigeminal ganglion of the rat. Light and electron microscopical observations. In *Pain in the Trigeminal Region* (ed. D. J. Anderson & B. Matthews), pp. 161–170. Amsterdam: Elsevier/North Holland.
- BERKHOUDT H (1980) The morphology and distribution of cutaneous mechanoreceptors (Herbst and Grandry corpuscles) in bill and tongue of the mallard (*Anas platyrhynchos* L.). *Netherlands Journal of Zoology* **30**, 1–34.
- BREWARD J, GENTLE MJ (1985) Neuroma formation and abnormal afferent nerve discharges after partial beak manipulation (beak trimming) in poultry. *Experientia* **41**, 1132–1134.
- CHIAIA NL, ALLEN Z, CARLSON E, MACDONALD G, RHOADES RW (1988) Neonatal infraorbital nerve transection in rat results in peripheral trigeminal sprouting. *Journal of Comparative Neurology* **274**, 101–114.
- DESSERICH M, ZISWILER V, FÖLSCH DW (1983) Die sensorische Versorgung des Hühnerschnabels. *Revue Suisse de Zoologie* **90**, 799–807.
- DESSERICH M, FÖLSCH DW, ZISWILER V (1984) Das Schanbelkupieren bei Hühnern. Ein Eingriff im innervierten Bereich. *Tierärztliche Praxis* **12**, 191–202.
- DUBBELDAM JL (1993) Systema nervosum periphericum. In *Handbook of Avian Anatomy: Nomina Anatomica Avium*, 2nd edn (ed. J. J. Baumel), pp. 555–584. Cambridge, Mass: Publications of the Nuttall Ornithological Club, no. 23.
- DUBBELDAM JL, BOUT RG, DE BAKKER MAG (1993) An analysis of the trigeminal branches in the beak of normal chicken and after beak trimming, with some behavioral observations. *European Journal of Neuroscience* (Suppl. 6), 57.
- DUBBELDAM JL, DEN BOER-VISSER AM (1993) Immunohistochemical localization of substance P, enkephalin and serotonin in the brainstem of chicken, with emphasis on the sensory trigeminal system. *Verhandlungen der Anatomischen Gesellschaft* **88**, 7–8.
- FRAHER JP (1989) Axon–myelin relationships in rat cranial nerves III, IV and VI: a morphometric study of large- and small-fibre classes. *Journal of Comparative Neurology* **286**, 384–390.
- FRAHER JP, O'LEARY D, MORAN MA, COLE M, KING RHM, THOMAS PK (1990) Relative growth and maturation of axon size and myelin thickness in the tibial nerve of the rat. I. Normal animals. *Acta Neuropathologica* **79**, 364–374.
- FRIED K & DEVOR M (1988) End-structure of afferent axons injured in the peripheral and central nervous system. *Somatosensory and Motor Research* **6**, 79–99.
- FRIED K, ARVIDSSON J, ROBERTSON B, PHALLER K (1991) Anterograde horseradish peroxidase tracing and immunohistochemistry of trigeminal ganglion tooth pulp neurons after dental nerve lesions in the rat. *Neuroscience* **43**, 269–278.
- GENTLE MJ (1989) Cutaneous sensory afferents recorded from the nervus intramandibularis of *Gallus gallus* var *domesticus*. *Journal of Comparative Physiology* **164**, 763–774.
- GENTLE MJ, BREWARD J (1986) The bill tip organ of the chicken (*Gallus gallus* var. *domesticus*). *Journal of Anatomy* **145**, 79–85.
- GOTTSCHALDT K-M (1985) Structure and function of avian somatosensory receptors. In *Form and Function in Birds*, vol. 3 (ed. A. S. King & J. McLelland), pp. 375–561. London: Academic Press.
- HIMES BT, TESSLER A (1989) Death of some dorsal root ganglion

- cells and plasticity of others following sciatic nerve sections in adult and neonatal rats. *Journal of Comparative Neurology* **284**, 215–230.
- HOLLAND GR, ROBINSON PP (1990) Cell counts in the trigeminal ganglion of the cat after inferior alveolar nerve injuries. *Journal of Anatomy* **71**, 179–186.
- LISNEY SJW (1989) Regeneration of unmyelinated axons after injury of mammalian peripheral nerve. *Quarterly Journal of Experimental Physiology* **74**, 757–784.
- RENEHAN WE, KLEIN BG, CHIAIA NL, JACQUIN MF, RHOADES RW (1989) Physiological and anatomical consequences of infraorbital nerve transection in the trigeminal ganglion and trigeminal spinal tract of the adult rat. *Journal of Neuroscience* **9**, 548–557.
- SAVY C, MARGULES S, FARKAS-BARGETON E, VERLEY R (1981) A morphometric study of mouse trigeminal ganglion after unilateral destruction of vibrissae follicles at birth. *Brain Research* **217**, 265–277.
- VAN ROOYEN J (1990) Schnabelkupieren und Fressverhalten. *Ethologentreffen* **3**, abstract 12.
- VAN ROOYEN J, BLOKHUIS H (1990) The quality of beak trimming. *Proceeding of the Society for Veterinary Ethology* (Pistoia, Italy), 115.
- YOUNG RF (1977) Fiber spectrum of the trigeminal sensory root of frog, cat and man determined by electronmicroscopy. In *Pain in the Trigeminal Region* (ed. D. J. Anderson & B. Matthews), pp. 137–147. Amsterdam: Elsevier/North-Holland.